Habitat Assessment Manual

DECRYPT Project

Integrative Taxonomy Framework

Фонд за науку
Републике Србије

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1. Purpose of the manual

This manual provides standardized guidelines for habitat assessment and ecological data collection of planthopper *Hyalesthes obsoletus* populations within the DECRYPT project framework. The protocol ensures that ecological information is collected in a consistent, reproducible, and scientifically rigorous manner across all sampling sites in Serbia. Standardization is essential for reliable cross-site comparisons and for integrating ecological variables with morphometric and genetic datasets.

The primary aim is to ensure comparable high-quality ecological data collection across all sampling sites to support:

- Morphological Functional Trait (MFT) analyses;
- Ecological Niche Divergence (EcoNDiv) modeling;
- Integrative taxonomic synthesis.

Habitat data are critical for testing whether host-plant-associated populations represent ecologically divergent lineages and potentially cryptic species.

2. Ecological context within DECRYPT

Hyalesthes obsoletus is a major vector of plant pathogenic bacterium '*Candidatus* Phytoplasma solani' and plays a significant role in the epidemiology of stolbur diseases in European agroecosystems. Understanding its ecological differentiation is therefore directly linked to improving pest management strategies.

The species is associated in Serbia with:

- *Urtica dioica;*
- *Convolvulus arvensis;*
- *Crepis foetida.*

Previous research has revealed genetic divergence among host-associated populations. DECRYPT integrates ecological niche parameters with morphometric and functional data to assess whether ecological differentiation supports species boundaries.

Habitat assessment therefore serves to:

- Quantify environmental parameters at collection sites;
- Identify landscape and microhabitat drivers of divergence;
- Provide variables for multivariate ecological modeling.

3. Standardized Habitat Assessment Protocol (HAP)

All fieldwork must follow the standardized HAP form. The protocol was specifically developed to capture ecological variables relevant to niche modeling and functional trait interpretation. Each sampling event must include complete documentation to ensure traceability, reproducibility, and compatibility with GIS-based analyses.

3.1 Administrative data

Accurate metadata are fundamental for spatial analyses and data integration. Incomplete or imprecise administrative data reduce the analytical value of ecological records.

Record the following:

- Collector (initials/full name);
- Date;
- Locality name;
- GPS coordinates (WGS84 format);
- Sample Code (Host plant initials + sample number + collector initials);
- Protocol Tracking Number (PTN).

GPS precision should be ≤5 m whenever possible to allow accurate landscape analyses.

4. Landscape-level assessment

Landscape context strongly influences host plant availability, dispersal potential, and population structure of *H. obsoletus*. Recording landscape characteristics allows later quantification of habitat heterogeneity and anthropogenic influence.

4.1 Type of Linear Landscape Element (LLE)

Indicate:

- Road verge;
- Field margin;
- Ditch.

Linear Landscape Elements function as semi-natural corridors within agricultural matrices. They often serve as refugia for host plants and may act as stable habitats for vector populations.

4.2 Adjacent and surrounding crops

Landscape matrix composition influences vector dispersal, phytoplasma transmission dynamics, and microclimatic conditions.

Indicate immediate bordering crop:

- Cereals;
- Sugar beet;
- Sunflower;
- Corn.

Indicate crops within visual range (landscape matrix context):

- Cereals;
- Sugar beet;
- Sunflower;
- Corn.

This information supports GIS-based EcoNDiv analysis using buffer zones (100 m, 500 m, 1 km) and allows quantification of land-use effects on ecological divergence.

5. Vegetation and host plant assessment

Microhabitat structure directly affects insect performance, feeding efficiency, predator exposure, and microclimatic buffering.

5.1 Host plant identification

Select host plant:

- *Urtica dioica;*
- *Convolvulus arvensis;*
- *Crepis foetida.*

Correct host identification is crucial because host association represents the core ecological axis of divergence tested in DECRYPT. Misidentification compromises following integrative analyses.

5.2 Host plant height

Measure height of three randomly selected individuals along the transect:

Example format:
45 cm, 52 cm, 48 cm

Host plant height reflects vegetation structure, competitive pressure, and local microclimate, all of which may influence insect functional traits and dispersal ability.

5.3 Vegetation structure

Indicate:

- Windbreak present;
- Single tree(s);
- Spontaneous shrubs;
- Only herbaceous vegetation.

Vegetation complexity may influence microclimate stability, predator and parasitoid communities, dispersal behavior and functional trait adaptation. Structural heterogeneity is an important explanatory variable in ecological niche modeling.

5.4 Management regime

Indicate whether LLE is:

- Cut;
- Grazed;
- Herbicide application;
- Burned;
- No management.

Management intensity affects host plant density, habitat continuity, disturbance frequency, insect population persistence. Understanding disturbance regimes is essential for interpreting population stability and potential ecological divergence.

6. Sampling strategy for *H. obsoletus*

Standardized sampling ensures comparability across host plants and sites and minimizes methodological bias.

6.1 Target period

Adult sampling must occur during the flight period (June–July). Sampling outside this window may bias population structure and underrepresent dispersing individuals.

6.2 Sampling method

- Entomological sweep net;
- Aspirator;
- Minimum disturbance of vegetation.

Sampling should aim to reduce habitat alteration and avoid selective collection bias.

6.3 Target sample size

Per host plant:

- 80 males;

- 80 females.

Specimens must be:

- Morphologically pre-identified;
- Preserved in 96% ethanol;
- Stored at 4°C.

Balanced sampling across sexes and host plants is necessary for robust morphometric and functional analyses.

7. Ecological variables for EcoNDiv analysis

Field data collected through HAP represent the fine-scale ecological basis of DECRYPT. These data will be integrated with broader environmental datasets to quantify niche divergence.

Integrated datasets include:

- Corine Land Cover (CLC v20u1);
- WorldClim bioclimatic variables (19 variables);
- Landscape composition buffers.

These datasets will allow:

- Multivariate niche comparison;
- PCA and PERMANOVA analyses;
- Environmental clustering of host-associated populations.

8. Data Quality Control

High-quality ecological data are foundational for integrative taxonomy. Even small recording errors can distort multivariate analyses.

To ensure consistency:

- Always complete all HAP fields;
- Avoid subjective descriptions (use predefined categories);
- Double-check GPS coordinates before leaving site;
- Take habitat photographs (4 cardinal directions + host close-up recommended);
- Record unusual conditions in the “Notes” section.

Incomplete habitat data compromise ecological modeling accuracy and reproducibility.

9. Integration with morphological and functional analyses

Habitat data do not stand alone, they are analytically integrated with morphological and genetic datasets to resolve species boundaries.

9.1 Morphological Functional Trait (MFT) analyses

Environmental variables will be tested against:

- Forewing length;
- Body size;
- Rostrum length;
- Hind leg dimensions.

Functional traits may reflect ecological adaptation to specific host-plant environments.

9.2 Geometric Morphometrics (GM)

Microhabitat divergence may correspond with:

- Wing shape variation;
- Male genitalia differentiation.

GM enables detection of subtle morphological differentiation potentially linked to ecological specialization.

9.3 Integrative taxonomic synthesis

The final DECRYPT objective is multimodal data integration (genetic + ecological + functional + micro-morphological).

Habitat data are foundational for answering:

Does *Hyalesthes obsoletus* represent a cryptic species complex?

10. Ethical and Environmental Considerations

Fieldwork must follow responsible scientific and environmental conduct. The project does not involve vertebrates or protected species.

Fieldwork principles:

- Minimize vegetation disturbance;
- Avoid over-collecting beyond required sample sizes;
- Respect agricultural land and landowners;
- Follow national biodiversity regulations.

11. Final Remarks

The standardized Habitat Assessment Protocol ensures:

- Reproducibility;
- Cross-site comparability;
- Robust ecological modeling;
- Strong integrative taxonomic inference.

Accurate habitat documentation is as critical as specimen collection. The ecological data collected today will directly influence the resolution of species boundaries and future pest management strategies in Serbia and across Europe.

Acknowledgment of Authorship

This Habitat Assessment Manual was developed exclusively for internal use by the DECRYPT project team within the framework of the Project *Integrating Morphometric and Ecological Niche Analyses to Define Species Boundaries in the planthopper Hyalesthes obsoletus Complex*, funded by the Science Fund of the Republic of Serbia, Grant no. 208.

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The protocol reflects the integrative methodological approach of the DECRYPT project, combining ecological field assessment, functional trait analysis, and geometric morphometrics to support taxonomic and ecological inference.